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**Vaccination of Chickens Against Viral
Arthritis/Tenosynovitis and
Reovirus-Induced Malabsorption Syndrome**

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ABSTRACT

Research experiments were conducted in Israel and the United States to determine whether or not avian reovirus vaccines, both live and inactivated, could produce sufficient immunity to protect chickens and their progeny against avian reovirus infection. In the case of viral arthritis/tenosynovitis, a distinct relationship can be demonstrated between avian reovirus infection and the clinical disease.

Tenosynovitis lesions do yield reovirus isolates and one can reproduce tenosynovitis by infection of chickens with reovirus isolates. In the case of malabsorption syndrome, the etiology is much more multifactorial, and avian reovirus is just one of the many viral and bacterial agents held responsible for the development of malabsorption syndrome. Reovirus isolates and serum antibodies from Israel and the United States have been demonstrated to cross-react to full serologic identity to the S1133 prototype.

Vaccination experiments described in this report show the distinction described above. Protection against development of tenosynovitis lesions after reovirus challenge could clearly be demonstrated in progeny of vaccinated breeder chickens, both in broiler as well as White Leghorn (WL) breeds. However, depressed weight gain, elevated alkaline phosphatase, or other parameters of malabsorption syndrome were not appreciably different in immune versus non-immune birds, again demonstrating a lack of evidence of reovirus etiology in the case of malabsorption syndrome.

It is concluded from the experiments reported that vaccination of broiler and WL breeders with inactivated reovirus vaccines, especially if a live reovirus priming vaccination is used, gives high serum antibody titers and good subsequent protection of the progeny against challenge infection.

OBJECTIVES OF ORIGINAL PROPOSAL:

The objectives of the original proposal were as follows. There were no changes or deviations from the stated objectives.

- I. To study the incidence of avian reovirus infections in cases of viral arthritis/tenosynovitis in broilers and turkeys in the United States and Israel and to determine, by laboratory techniques (virus neutralization testing), whether reovirus isolates from both countries are serotypically similar or even identical.
- II. To study the role of avian reovirus vaccines in the possible control of viral arthritis/tenosynovitis, both directly by the use of fully attenuated, apathogenic live vaccines in young birds or by production of maternal immunity in progeny of breeders by vaccination of breeders at mature age with live modified reovirus vaccines or a combination of vaccination with live modified vaccine followed by a vaccination with inactivated oil adjuvant vaccine. If reovirus isolates from chickens and turkeys in Israel and the U.S. are serotypically compatible, chances are that vaccination will be an effective control method for viral arthritis/tenosynovitis in Israel, as has been demonstrated in U.S.
- III. To study the incidence of malabsorption syndrome ("femoral head necrosis"/ "brittle bone disease") in broilers in Israel and the U.S., clinically and histopathologically as well as by virological means, to determine the etiological role of avian reovirus in this disease and determine possible serotypical relationships between reovirus isolates from broilers in Israel and the U.S.
- IV. To study the role of vaccination in the control of malabsorption syndrome by laboratory and field trials.
- V. To study the role of immunosuppression from concurrent infections with infectious bursal disease, on the incidence and the severity of malabsorption syndrome.

INTRODUCTION:

Experiments have been conducted in the United States and Israel with vaccination of broiler breeders against avian reovirus-induced viral arthritis/tenosynovitis, and malabsorption syndrome. The role of avian reovirus in viral arthritis/tenosynovitis has been clearly established; virus isolations from lesions of tenosynovitis yielded reovirus, and it was invariably possible to reproduce typical lesions of tenosynovitis with reovirus isolates.

In the case of malabsorption syndrome the etiological role of avian reovirus has not been clearly identifiable. On the one hand, avian reovirus could be isolated from intestinal contents, liver, or bone marrow tissues of chickens with typical signs of malabsorption syndrome. However, several other viruses also were isolated at times from such lesions. These other viruses included parvovirus, adenovirus, calicivirus, enterovirus, and rotavirus. Furthermore, when reovirus isolates were used to reproduce typical lesions of malabsorption syndrome, such as enteritis, pale birds, "femoral head necrosis" (osteoporosis), stunting, abnormal feathering ("helicopter" birds), the attempts were frequently unsuccessful or only partially successful.

Interaction of avian reovirus and infectious bursal disease by concurrent experimental infections of chickens with both viruses did indicate an aggravating effect upon body weight increase, but not on the incidence or gravity of microscopic tenosynovitis lesions (Table 1).

Experiments Conducted in the United States (L. van der Heide):

Experiments were conducted with vaccination of groups of 23 SPF White Leghorn (WL) or commercial broiler breeders (20 hens; 3 males) using inactivated reovirus vaccines with or without a priming vaccination with live attenuated reovirus vaccines.

Four commercially available inactivated reovirus vaccines were used: 1) CO₈ reovirus vaccine; Merieux Labs, Athens, Georgia: 2) Intervet reovac; Intervet International, Millsboro, Delaware: 3) MBL-reovaccine; Maine Biological Lab, Waterville, Maine: and 4) Salsbury-reovaccine; Salsbury Labs, Charles City, Iowa. Priming vaccination was administered as pullets, at 11 weeks of age with VA-Vac, reovirus vaccine, live (Salsbury Labs). Unvaccinated breeder chickens also were kept as controls.

Hatching eggs were collected from vaccinated and unvaccinated breeder chickens and two or three groups of eggs were hatched consecutively, as indicated in Tables 2, 3, 4, and 5.

Groups of 15 to 20 progeny chicks from each of the vaccinated breeder groups were experimentally infected by oral (WL) or subcutaneous (commercial broiler breeders) route with approximately 10^4 ELD₅₀ of avian reovirus, strain S1133, 14th embryo passage.

At 28 days of age, all birds were bled and killed. The birds were weighed; their blood serum examined for reovirus antibodies by agar gel precipitation (AGP) test, for alkaline phosphatase serum levels by spectrophotometric assay (Sigma, Inc., St. Louis, Missouri).

Cross sections of digital flexor tendons were fixed in 10% formalin-saline and paraffin embedded. Microtome sections of 5 microns thickness were cut and stained with hematoxylin-eosin (HE) for microscopic examination for lesions of tenosynovitis. The results of the experiments are compiled in Tables 2 through 7.

Results clearly indicate that the use of a live primer vaccination followed inactivated reovirus vaccine in breeder chickens gives better protection of progeny chicks against reovirus challenge than the use of inactivated vaccine only.

We have conducted several experiments in commercial broiler and SPF WL chickens, using concurrent oral infection with a pathogenic strain of infectious bursal disease virus (IBDV) and subcutaneous infection with avian reovirus strain L-50, which has been shown previously to reproduce malabsorption syndrome in chickens.

Although it was obvious that the degree of proventricular and intestinal lesions was more severe after concurrent IBDV and reovirus infections, as compared to single reovirus or IBDV infections, the considerable variability that was encountered in this histologic study seems to indicate that such lesion examination might not be a reliable parameter of such infections. Neither was the incidence of femoral head necrosis (osteoporosis) a consistent feature.

Several experiments were conducted with reovirus infections with and without concurrent IBDV infections. Groups of 20 or 30 commercial broiler chickens, that possessed maternal antibodies against reovirus and IBDV at day-old, were infected at 1-day of age by subcutaneous (sc) route with 2 or 3 strains of reovirus: S1133 (tenosynovitis virus isolate, University of Connecticut; van der Heide); CO₈ malabsorption syndrome virus isolate, University of Georgia, Villegas); and L-50 malabsorption syndrome virus isolate, University of Connecticut; van der Heide). At 12 days of age the chickens were infected sc

with IBDV. At 25 days (Experiment 89) or 27 days of age (Experiment 90) the chickens were bled, killed, and weighed.

Digital flexor tendon tissues were collected and fixed in formalin and further processed for microscopic examination. Tenosynovitis lesions were scored as 0 (no lesions), + (minimal lymphocytic infiltration in tendon sheaths), ++ marked infiltration and serous exudate in tendon sheaths); +++ (extensive lymphocytic infiltration), and ++++ (maximum lesions with extensive exudation and infiltrates with lymphocytes and heterophils).

Blood serum samples from the experimental chickens were tested for the presence of antibodies to reo- and IBD virus by agar gel precipitin (AGP) testing. At 7 and 12 days of age, a general mean titer was determined against IBDV by the micro- virus neutralization test.

Blood serum samples were frozen at 0°F and further examined for the level of serum alkaline phosphatase, using procedures outlined by Vertommen et al. (Avian Pathology, 1980; 9:133-142). Results are given in Tables 8 and 9.

It appears that at 25 or 27 days of age in these two experiments, the mean lesion score of tenosynovitis was not appreciably different whether the birds were infected with reovirus alone, or in combination with IBDV. In fact, in Experiment 89, the lesion score seemed lower after combined reo- and IBD virus infection than after reovirus infection alone. This is possibly caused by the immunosuppressive effect of IBDV infection which would decrease the degree of lymphocytic infiltration into the tendon sheaths. However, it appears that concurrent IBDV infection does not significantly change the tenosynovitis response of reovirus infection.

The decreased weight gain, which is commonly observed in chickens after reovirus infection (van der Heide et al.: Avian Diseases, 1974; 18: 289-296) was not more severe after concurrent reo-IBD virus infection, compared to single reovirus infection.

It was observed that average alkaline phosphatase levels were lower at 27 days of age in birds infected with reovirus compared to the uninfected controls, although great variation was found between individual samples. A subsequent test of alkaline phosphatase levels in reovirus-infected chickens at 4 and 12 days of age (results given in Tables 10 and 11) seems to

indicate that increased levels of alkaline phosphatase can be found at 12 days of age, while at 27 days of age there exists a compensatory decrease in alkaline phosphatase levels. This phenomenon has also been observed in Holland (Jaspers et al., March 4-6, 1985; Proc. Western Poultry Disease Conf., Davis, California). It is also interesting to note that the alkaline phosphatase levels are higher at 12 days of age in chickens infected with strains of reovirus of malabsorption type (L-50 and CO₈) than after infection with tenosynovitis-type reovirus (S1133) (see Tables 10 and 11). The uninoculated control birds usually have an alkaline phosphatase level at 12 days of age of approximately 2700 U/L. The level in Table 10 of 7796 U/L is probably a test error.

In addition to the data presented here that seem to indicate a difference in biotype between the S1133 (tenosynovitis) and CO₈ (malabsorption) strains of reovirus, we have conducted further experiments in collaboration with Dr. Paul Goetinck of the Department of Animal Genetics at the University of Connecticut. These studies indicate significant differences in certain blood components in chickens infected with S1133 strain reovirus as compared to chickens infected with CO₈ strain reovirus, again indicating biotype differences between the two viruses.

Serologically, however, the two virus strains are very closely related, so that vaccination experiments showed good cross protection between the various reovirus strains.

In conclusion there is histologic indication that IBDV infection can enhance the gravity of a reovirus infection. This effect was observed in gastrointestinal tissues and not in tendon sheath tissues. This finding is in agreement with several field observations that improved breeder vaccination against IBDV with subsequent increasing levels of maternal immunity against IBDV in their broiler progeny results in a dramatic decrease in the incidence and degree of malabsorption syndrome in the broilers. It was not possible, however, in an experimental setting to demonstrate significant differences in body weights or alkaline phosphatase levels in chickens after reovirus infection with or without concurrent IBDV infection. There appeared to be an influence of reovirus biotype on the level of increase in alkaline phosphatase. However, all reovirus isolates used thus far have been capable of inducing tenosynovitis lesions, whether they were isolated from tenosynovitis or malabsorption syndrome lesions.

Experiments Conducted in Israel (Y. Weisman):

Experiments on vaccination of broiler breeders with inactivated reovirus vaccines were conducted. Two commercial, inactivated, oil-emulsion reovirus vaccines were used. One has strains S1133 and 1733 (Maine Biological Labs (MBL), Waterville, Maine, USA) and the other vaccine has strains 1733 and 2408 (Intervet Labs, Millsboro, Delaware, USA).

One group of broiler breeders was vaccinated with MBL vaccine, 0.5 ml sc per bird; a second group was vaccinated with Intervet vaccine, 0.5 ml sc per bird; and a third group was kept as non-vaccinated controls. The breeder chickens were bled at 15-day intervals and blood serum was examined by the AGP test and the enzyme-linked immunosorbent assay (ELISA) for antibodies against reovirus, until 9 months postvaccination.

Progeny chicks were hatched from eggs laid by these three groups of breeders and the progeny chicks were blood tested at 1-day of age in weekly hatches, up to 7 months postvaccination of the breeders. The results of breeder serum testing are given in Tables 12-14 and the results of progeny serum testing are given in Tables 15-17.

In two hatches (3rd and 5th) of progeny chicks from the three groups of breeders, the chicks were kept in separate rooms for a period of 28 days. Blood samples were taken from the chicks at 0, 14, 21, and 28 days of age and the sera tested by AGP and ELISA. Results are shown in Tables 18 and 19. Results indicate that good serum immunity levels can be found by AGP as well as by ELISA testing up until 6 months postvaccination (MBL vaccine, Group 1) after which the ELISA serum values began to decrease below a level that can be considered highly immune. In Group 2 (Intervet vaccine), the ELISA serum values are highly adequate until 8 months postvaccination.

The incidence of AGP reactors to reovirus antigen appear to remain high during the entire experimental period of 10 months postvaccination. Some evidence of field exposure to reovirus is evident in the non-vaccinated breeders.

Field exposure to reovirus is very common in the entire world, as reoviruses are ubiquitous globally. Such an exposure can be used as an advantage because it acts as a priming vaccination for the subsequent inactivated oil-emulsion vaccine administration. However, if clinical tenosynovitis could result in the breeders from such early field exposure, the necessity of early vaccination (e.g., 7 days of age) with highly attenuated apathogenic reovirus vaccine becomes a must.

In spite of the field exposure to reovirus, the non-vaccinated breeders (Table 14) do not have adequate ELISA serum values at any given test time. It is the inactivated reovirus vaccine that causes the presence of the high ELISA serum values of reovirus antibody in Groups 1 and 2.

Progeny chicks of the two vaccinated breeder groups have good reovirus antibody levels at 1-day of age, as evidenced in Tables 15 and 16. ELISA serum values are generally above acceptable levels for day-old progeny, although higher in Group 2 than in Group 1. Progeny of Group 3 (non-vaccinated controls) were consistently negative in the AGP test at 1-day of age, and the ELISA serum values were generally below 2.4, which is considered low (Table 17). When progeny chicks of the three groups of breeders were tested for reovirus antibody during 4 weeks of life (Tables 18 and 19), one can conclude that after 21 days of age maternal immunity is waning.

If field exposure to reovirus is evident in commercial poultry operations, an adequate level of maternal immunity can protect the chickens against early infection. In particular, the first two weeks of life are critical in this respect.

Description of Cooperation:

Dr. Yoram Weisman, Kimron Veterinary Institute, the cooperating agency in Israel, has vaccinated groups of 20-week-old breeder chickens with either one of two commercial inactivated oil-emulsion reovirus vaccines (Maine Biological Labs, Waterville, Maine, USA, and Intervet Labs, Millsboro, Delaware, USA), preceded by live virus vaccination at 8 and 17-18 weeks of age. The breeder flocks were bled at intervals, as indicated, and serum antibody levels against reovirus tested by AGP and ELISA tests.

Progeny chicks were hatched from two vaccinated and one unvaccinated control breeder flocks and serologically tested by AGP and ELISA tests at 1, 8, 14, and 27 days of age to determine the longevity of maternal anti-reovirus immunity. The results are shown in Tables 20 and 21.

The results clearly indicate the presence of high levels of antibody against reovirus in the breeder serum samples at 2, 4, 6, 10, and 14 weeks postvaccination. ELISA titers of greater than 4 are evidence of excellent immunity against reovirus as late as 14 weeks postvaccination. The unvaccinated control flock had negative titers of 2.65 ± 0.37 at the same time.

Progeny chicks had maternal antibody level against reovirus until 14 days

of age. The results of the experiments in the United States have shown that although maternal antibody levels are not high in a VN or ELISA test, adequate protection is afforded to such progeny chicks against challenge.

The duration of measurable maternal immunity until 14 days of age is adequate, since age resistance to oral challenge with reovirus after 14 days of age has been shown in our laboratory.

Evaluation of Research Achievements:

The research described in this report has achieved four major goals as set out in the research proposal:

1. It was clearly demonstrated that viral arthritis/tenosynovitis is caused by avian reovirus, and also that reovirus isolates from tendon tissues shared the same or similar serotype. This is important from the standpoint of protection, where the existing commercial reovirus vaccines, which share the major serotype (S1133) will protect against most, if not all of the existing field infections with avian reovirus.

2. The relationship of malabsorption syndrome and avian reovirus was found not to be so clear as in viral arthritis/tenosynovitis. On the contrary, all data indicate the complex multifactorial nature of malabsorption syndrome and also that reovirus vaccination is not a satisfactory prevention measure against all forms of malabsorption syndrome.

3. Protection against viral arthritis/tenosynovitis is afforded to the progeny of reovirus vaccinated breeders. A vaccination regimen of broiler breeders with a live reovirus vaccine primer, followed by administration of an inactivated oil emulsion reovirus vaccine is clearly superior in affording progeny protection to the use of inactivated vaccine only. Protection of progeny against reovirus-induced viral arthritis/tenosynovitis was also demonstrated in White Leghorn egg-laying type of chickens.

4. Concurrent infection of chickens with reovirus and infectious bursal disease (IBD) virus demonstrated an aggravation of lesion development. As such, the preventive vaccination of breeder chickens against IBD is indicated to decrease the degree of disease in viral arthritis/tenosynovitis and even some cases of malabsorption syndrome.

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TABLE 1

Experiment #87

Four groups of SPF-White Leghorn chickens were infected S.C. at 1 day of age with reovirus strain L-50 (isolated from intestines of 4 day-old chicks with malabsorption syndrome) and/or at 12 days of age orally with Infectious Bursal Disease Virus (IBDV).

All chickens were bled, killed and weighed at 30 days of age.

	No. birds	Weight per chicken in grams	AGP reovirus	AGP IBDV	N.I. reovirus	Microscopic tendon lesions
Group 1: Not infected	9	230	0/9	0/9	0.0	0/18
Group 2: Infected reovirus S.C. $10^{6.8}$ ELD/ chick at 1 day of age	20	180	20/20	0/20	1.7	39/40
Group 3: Infected IBD orally $10^{3.5}$ EDL per chick at 12 days of age	10	200	0/10	10/10	0.0	0/20
Group 4: Infected reovirus 1 day S.C. and IBDV 12 days orally	20	160	20/20	20/20	1.9	34/36

Microscopic lesions of tenosynovitis were severe in reovirus-infected birds, whether co-infected with IBDV or not.

Weight gain was depressed in reovirus-infected birds, but particularly in orally infected (reovirus + IBDV) birds.

Histological sections of liver, spleen, heart, kidneys, bursa, thymus, proventriculus, pancreas and duodenum were examined microscopically.

No lesions were observed in thymus or bursa of reovirus-infected birds. IBDV-infected birds had bursal lesions. Intestinal, pancreatic and proventricular lesions were not significant over uninfected control birds, except in some IBDV-infected chickens.

This observation indicated the inconsistent development of specific lesions of malabsorption in reovirus-infected birds. Yet decreased weight gain was observed more in reovirus and reovirus + IBDV infected birds than in IBDV infected birds or uninfected controls. Also, tenosynovitis lesions were consistently found in reovirus and reovirus + IBDV infected birds, even if the reovirus was from malabsorption cases.

TABLE 2. Serological response of breeder chickens after vaccination with live and/or inactivated reovirus vaccines.

	Pen	AGP S1133	Virus neutralization				ELISA Agritech ³
			S1133		CO _B		
			α^1	β^2	α	β	
Preinoculation	1	0/21	1.0	2.1	1.5	4.0	ND ⁴
	2	0/21	0.7	2.1	1.1	4.0	ND
	3	0/20	1.0	2.0	1.3	4.0	ND
	4	0/20	0.8	2.5	0.9	4.0	ND
3 weeks postvaccination	1	0/21	1.2	4.0	1.3	4.0	0.44
	2	0/21	1.2	4.0	0.9	4.0	0.27
	3	0/20	2.1	4.9	2.0	4.0	0.92
	4	0/20	1.6	4.9	2.0	4.0	1.24
7 weeks postvaccination	1	2/21	2.6	4.6	2.3	4.0	0.82
	2	3/21	2.6	11.0	2.4	5.3	2.55
	3	8/19	3.1	21.0	3.0	9.8	3.01
	4	10/19	3.2	11.0	3.3	4.6	1.39
13 weeks postvaccination	1	8/20	2.6	4.6	2.9	8.0	2.14
	2	13/20	2.8	5.7	3.0	6.5	2.57
	3	19/20	2.9	5.7	2.7	10.6	2.98
	4	18/18	2.8	8.0	2.9	34.0	2.82

¹ α method of virus neutralization test, GMT titer.

² β method of virus neutralization test, average titer.

³ELISA test using Agritech plates, $\frac{\text{sample OD} - \text{negative OD}}{\text{positive OD} - \text{negative OD}}$

For vaccination histories of Pens 1 through 4, see Legend, Table 3.

TABLE 3. Progeny Challenge Data

Group	Status	Pen	AGP S1133	Alkaline phosphatase (avg)	Bird weight (grams)	Histo- pathologic lesions	Tests at day-old					
							AGP S1133	Virus neutralization				ELISA (Agritech)
								S1133 a	CO ₂ β	S1133 a	CO ₂ β	
I	Not challenged	SH	7/9	1,802	219	0/18	1/11	1.0	4.0	1.2	4.0	0.09
		1	5/8	2,801	188	0/16	1/10	1.1	4.0	1.2	4.0	0.13
		2	0/10	834	204	0/20	0/5	1.2	4.0	1.1	4.0	0.07
		3	1/10	1,508	206	0/20	0/6	1.9	4.0	2.1	4.0	1.07
		4	1/9	975	209	0/16	0/4	2.8	4.0	2.9	4.0	2.35
II	Not challenged	SH	1/16	1,734	167	0/32	0/7	1.3	4.0	0.2	4.0	0.07
		1	0/8	2,150	187	0/13	3/11	1.3	4.0	1.7	4.0	0.07
		2	0/10	1,954	194	0/20	4/9	2.3	4.0	1.9	4.0	2.47
		3	1/9	2,082	175	0/18	4/12	2.2	4.0	2.0	4.6	2.11
		4	1/4	1,397	229	0/8	0/0	2.0	4.0	2.3	4.0	0.70
I-a	Challenged	SH	6/7	4,088	179	4/14						
		1	13/14	1,432	183	12/28						
		2	2/29	1,763	219	1/18						
		3	3/26	719	199	3/52						
		4	3/13	942	204	1/26						
II-a	Challenged	SH	10/15	1,870	168	6/22						
		1	13/16	3,120	173	3/31						
		2	1/14	2,124	191	0/28						
		3	3/11	2,115	187	1/22						
		4	2/10	2,495	218	2/22						
I-b	Challenged	SH	15/15	1,231	202	8/30						
		1	15/15	873	184	9/30						
		2	8/8	908	222	0/10						
		3	16/25	1,145	198	4/54						
		4	1/13	1,420	206	2/26						
II-b	Challenged	SH	7/16	2,528	161	6/30						
		1	3/15	2,059	181	3/30						
		2	0/17	1,843	177	2/27						
		3	4/9	2,121	193	0/18						
		4	1/10	1,397	188	0/20						

Groups I and II: Pen SH, unvaccinated, not challenged
 1, CO₂ killed vaccine, not challenged
 2, ICB killed vaccine, not challenged
 3, live vaccine and killed ICB vaccine, not challenged
 4, live vaccine and killed CO₂ vaccine, not challenged

Groups I-a and II-a: As above, except that all were challenged with CO₂

Groups I-b and II-b: As above, except that all were challenged with S1133

TABLE 4. Serological response of breeder chickens after vaccination with live and/or inactivated reovirus vaccines. Flock 93.

	Pen	AGP S1133	Virus neutralization				ELISA	
			S1133		CO8		Agritech ³	SPF ⁴
			α^1	β^2	α	β		
Preinoculation	1	0/18	0.8	4.3	1.1	4.0	0.21	1.16
	2	0/18	1.0	4.3	1.1	4.0	0.15	0.98
	3	0/18	1.0	4.3	1.1	4.0	0.19	1.01
	4	0/18	1.2	4.3	1.1	4.0	0.01	0.68
2 weeks postvaccination	1	1/18	2.0		2.0		0.81	0.45
	2	4/18	2.3		2.2		0.87	0.46
	3	1/18	1.8		1.7		1.18	0.45
	4	1/18	2.4		2.2		1.10	0.44
8 weeks postvaccination	1	5/18	3.8		3.4		1.94	0.93
	2	12/18	3.7		2.9		2.81	0.91
	3	4/18	2.8		2.4		1.76	0.85
	4	7/16	3.4		3.2		2.62	1.20
13 weeks postvaccination	1	5/18	3.9		3.6		2.38	1.26
	2	9/18	3.6		3.1		2.72	1.14
	3	1/18	2.9		2.6		2.10	1.22
	4	5/16	3.9		3.4		2.40	1.35

¹ α method of virus neutralization test, GMT titer.

² β method of virus neutralization test, average titer.

³ELISA test using Agritech plates, $\frac{\text{sample OD} - \text{negative OD}}{\text{positive OD} - \text{negative OD}}$

For vaccination histories of Pens SH and 1 through 4, see legend Table 3, Flock 93.

TABLE 5. Progeny Challenge Data. Flock 93.

Group	Status	Pen	AGP S1133	Alkaline phosphatase (avg)	Bird weight (grams)	Histo- pathologic lesions	Tests at day-old				
							AGP S1133	S1133 α	CO8 α	ELISA tests	
										Agritech	SPF
I	Not challenged	SH	0/11	2,086	209*	0/22	0/4	1.3	1.4	0.12	0.27
		1	1/6	3,250		0/12	3/7	1.6	2.5	1.38	0.37
		2	0/9	1,496		0/18	5/7	1.6	1.6	1.80	0.68
		3	0/3	4,897		0/5	1/6	1.5	1.6	1.66	0.51
		4	0/3	1,760		0/6	2/3	2.6	2.7	1.77	0.99
II	Not challenged	SH	0/9	1,240	200	0/18	0/4	0.6	0.7	0.00	0.26
		1	0/1	1,751	171	0/14	3/4	2.3	2.6	0.78	0.49
		2	0/3	1,368	213	0/6	1/4	2.5	2.3	0.66	0.38
		3	0/4	1,538	190	0/8	1/8	2.3	2.5	1.05	0.51
		4	0/3	2,313	180	0/6	0/2	2.0	2.1	0.51	0.76
I-a	Challenged	SH	9/15	1,214	229	5/30					
		1	3/13	1,223	229	0/22					
		2	3/10	1,075	204	0/24					
		3	0/7	3,509	220	0/18					
		4	0/7	1,078	197	0/12					
II-a	Challenged	SH	5/15	1,389	195	2/30					
		1	0/13	1,238	174	0/24					
		2	0/5	1,572	196	0/10					
		3	0/14	1,802	173	1/27					
		4	0/10	1,682	218	0/20					
I-b	Challenged	SH	15/15	1,024	201	4/30					
		1	1/8	1,504	233	0/16					
		2	0/12	1,590	215	0/24					
		3	6/6	1,729	227	0/12					
		4	2/5	2,356	232	0/10					
II-b	Challenged	SH	14/15	1,173	179	7/30					
		1	0/12	1,510	138	0/24					
		2	0/4	1,151	190	0/8					
		3	9/12	1,547	195	1/24					
		4	0/10	1,383	218	0/20					

Groups I and II: Pen SH, unvaccinated, not challenged

1, MBL killed vaccine, not challenged

2, Salsbury killed vaccine, not challenged

3, Live vaccine and killed Salsbury vaccine, not challenged

4, Live vaccine and killed MBL vaccine, not challenged

Groups I-a and II-a: As above except that all were challenged with CO₈

Groups I-b and II-b: As above, except that all were challenged with S1133.

TABLE 6

Progeny test 1

Weights, antibody and alkaline phosphatase level at 21 days of age in progeny of broiler breeders vaccinated (3 weeks p.v.) with two different inactivated reovirus vaccines, after day-old s.c. challenge with 3 strains of reovirus.

Vaccination of breeders	Challenge virus/dose	Average weight in grams/bird	AGP (ag 1133)	Alk. phosph. av. level of 10 in U/L
Live at 7 days and 10 weeks	Not challenged	338	0/8	4422
"	$CO_8 10^{4.0} ELD_{50}$	233	6/6	5968
"	$L50/10^{4.8} ELD_{50}$	278	8/9	5647
"	$1133/10^{4.3} ELD_{50}$	272	10/10	5105
Live at 7 days and 10 weeks inact. CO_8 at 22 weeks	Not challenged	302	0/10	4962
"	CO_8	308	15/15	5364
"	L50	317	0/15	3687
"	1133	253	11/11	4983
Live at 7 days and 10 weeks inact. 2408+1733 at 22 weeks	Not challenged	329	0/7	5040
"	CO_8	278	10/10	6040
"	L50	319	3/15	4734
"	1133	283	15/15	4681

TABLE 7

Progeny test 2

Weights, antibody and alkaline phosphatase level at 21 days of age in progeny of broiler breeders vaccinated (7 weeks p.v.) with two different inactivated reovirus vaccines, after day-old s.c. challenge with 3 strains of reovirus.

Vaccination of breeders	Challenge virus/dose	Average weight in grams/bird	AGP (ag 1133)	Alk. phosph. av. level of 10 in U/L
Live at 7 days and 10 weeks	Not challenged	307	0/15	3507
"	CO ₈ /10 ^{5.0} ELD ₅₀	374	11/13	5464
"	L50/10 ^{5.8} ELD ₅₀	328	11/20	4088
"	1133/10 ^{5.3} ELD ₅₀	290	15/15	4221
Live at 7 days and 10 weeks inact. CO ₈ at 22 weeks	Not challenged	281	0/15	4591
"	CO ₈	340	11/15	4518
"	L50	337	10/17	5670
"	1133	315	11/11	3962
Live at 7 days and 10 weeks inact. 2408+1733 at 22 weeks	Not challenged	327	0/17	4539
"	CO ₈	380	12/19	4428
"	L50	359	6/17	3978
"	1133	347	14/14	4591

Table 8.

Parameters at 25 days of age in commercial broiler chickens after day-old s.c. reovirus and s.c. IBDV infection at 12 days.

Group of chickens	AGP		Weight grams/chicken	Alkaline phosphatase U/L.	Microscopic lesions of tenosynovitis	
	S1133	IBD			#/total	lesion score average
Not infected	0/21	0/21	373	2821	1/24	0.02
S1133 reovirus	23/23	0/23	376	3406	46/46	3.85
C08 reovirus	20/20	0/20	408	2918	37/40	3.57
S1133 reovirus and IBDV	30/30	30/30	345	2336	60/60	3.22
C08 reovirus and IBDV	26/26	23/26	410	1554	47/52	3.44
1 day of age	0/10	0/10 GMT=256				
12 days of age not infected	0/5	0/5 GMT=9		1214		
12 days of age S1133 reovirus	5/5	0/5		640		
12 days of age C08 reovirus	5/5	0/5		500		

Table 9.

Parameters at 27 days of age in commercial broiler chickens after day-old s.c. reovirus and s.c. IBDV infection at 12 days.

Group of chickens	AGP		Weight grams/chicken	Alkaline phosphatase U/L.	Microscopic lesions of tenosynovitis	
	S1133	IBD			#/total	lesion score average
Not infected	0/24	0/24	631	2336	0/48	0.00
S1133 reovirus	24/24	0/24	514	1493	48/48	3.69
L50 reovirus	22/22	0/22	594	1300	41/44	3.14
C08 reovirus	11/11	0/11	432	1400	23/24	3.29
IBDV	0/22	1/22	589	2926	0/44	0.00
S1133 reovirus and IBDV	22/23	1/23	533	1730	46/46	3.93
L50 reovirus and IBDV	24/24	1/24	571	4237	47/48	2.62
C08 reovirus and IBDV	12/12	0/12	522	1840	24/24	3.92
1 day of age	4/20	20/20 GMT=1910				
12 days of age (not infected)	0/10	2/10 GMT=274				
12 days of age S1133 reovirus	9/10	4/10				
12 days of age L50 reovirus	3/10	6/10				
12 days of age C08 reovirus	8/8	2/8				

Table 10.

Alkaline phosphatase levels in bloodserum of commercial broiler chickens at 4, 12, and 27 days of age after day-old reovirus and 12 days-old IBDV infection.

Group of chickens	Alkaline phosphatase level in U/L.		
	4 days of age	12 days of age	27 days of age
Not infected	6439	7796	2336
SI133-reovirus	5490	4052	1493
L50-reovirus	5439	8212	1300
CO ₈ -reovirus	4076	5746	1400
IBDV and SI133 reovirus	N.D.	N.D.	1730
IBDV and L50 reovirus	N.D.	N.D.	4237
IBDV and CO ₈ reovirus	N.D.	N.D.	1340

Table 11.

Alkaline phosphatase levels in bloodserum of commercial broiler chickens at 4, and 12 days of age after day-old reovirus and 12 days-old IBDV infection.

Group of chickens	Alkaline phosphatase level in U/L.	
	4 days of age	12 days of age
Not infected	10493	2537
S1133-reovirus	5474	2699
L50-reovirus	4455	6504
CO ₃ -reovirus	9312	8098

TABLE 12. Serum antibodies against reovirus in broiler breeders vaccinated with inactivated reovirus vaccine. Group 1: 0.5 ml SC MBL.

ELISA (log 10)		AGP	Date of test
VA	IBDV		
32 (2.94±0.28)	31 (3.77±0.38)	16/32	4-8-84
34 (2.80±0.28)	34 (3.45±0.35)	33/34	4/24-84
32 (3.13±0.10)		30/32	5-9-84
22 (3.21±0.08); 8 (>3.37)	23 (3.96±0.35); 6 (>4.5)	30/30	5-24-84
29 (3.00±0.18)		24/29	6-24-84
29 (3.07±0.19)		22/29	7-24-84
29 (2.93±0.20)		26/29	8-20-84
26 (3.16±0.09); 3 (>3.37)		22/29	9-24-84
28 (3.22±0.07); 1 (>3.37)		26/29	10-24-84
28 (2.75±0.22)	15 (3.91±0.38)	21/28	11-26-84
28 (2.78±0.18)		20/28	12-24-84
26 (2.60±0.13)		20/26	1-23-85
		17/26	2-20-85

TABLE 13. Serum antibodies against reovirus in broiler breeders vaccinated with inactivated reovirus vaccine: Group 2: 0.5 ml SC Intervet

ELISA (log 10)			Date of test
VA	IBDV	AGP	
32 (2.70±0.21)	33 (3.61±0.40)	18/32	4-8-84
33 (2.95±0.21)	31 (3.63±0.34)	32/33	4-24-84
26 (3.20±0.13; 6 (>3.36)		30/32	5-9-84
8 (3.26±0.07); 23 (>3.36)	21 (>4.5); 13 (4.17±0.27)	30/31	5-24-84
19 (3.16±0.15); 12 (>3.36)		31/31	6-24-84
30 (3.18±0.15); 1 (>3.37)		26/31	7-24-84
30 (3.18±0.15); 1 (>3.37)		31/31	8-20-84
10 (3.27±0.08); 20 (>3.37)		23/30	9-24-84
18 (3.23±0.07); 12 (>3.37)		25/30	10-24-84
30 (3.01±0.20)	2 (>4.5); 13 (3.75±0.37)	24/30	11-26-84
29 (2.99±0.20)		25/29	12-24-84
29 (2.68±0.18)		24/29	1-23-85
		25/29	2-26-85

TABLE 14. Serum antibodies against reovirus in unvaccinated broiler breeders

ELISA (log 10)			AGP	Date of test
VA	IBDV			
1 (>2.35); 31 (2.64±0.20)	32 (3.55±0.46)		14/33	4-8-84
32 (2.69±0.21)	32 (3.70±0.41)		26/32	4-24-84
32 (2.67±0.17)			15/32	5-9-84
32 (2.58±0.18)	1 (>4.5); 31 (3.89±0.36)		0/32	5-24-84
1 (>2.35); 31 (2.59±0.19)			14/32	6-24-84
1 (>2.35); 31 (2.59±0.17)			12/32	7-24-84
31 (2.64±0.17)			8/31	8-20-84
30 (3.00±0.21)			0/30	9-14-84
29 (2.99±0.20)			0/29	10-25-84
24 (2.60±0.18)	15 (3.47±0.37)		0/30	11-26-84
29 (2.57±0.11)			1/29	12-24-84
4 (>2.35); 23 (2.42±0.05)			2/29	1-23-85
			1/29	2/26-85

TABLE 15. Serum antibodies against reovirus in day-old progeny from breeders vaccinated with MBL inactivated reovirus vaccine.

No.	Date of test	ELISA-VA	AGP
1	6-13-84	1 (<2.35); 23 (2.71±0.25)	13/24
2	6-20-84	2 (<2.35); 18 (2.45±0.08)	3/20
3	6-26-84	2 (<2.35); 18 (2.54±0.10)	6/20
5	7-11-84	5 (<2.35); 15 (2.43±0.09)	5/20
6	7-18-84	20 (2.74±0.21)	14/20
7	7-25-84	20 (2.50±0.11)	6/19
8	8-1-84	2 (<2.35); 16 (2.48±0.06)	4/18
9	8-8-84	2 (<2.35); 18 (2.45±0.08)	6/20
10	8-16-84	5 (<2.35); 15 (2.47±0.08)	4/20
11	8-22-84	6 (<2.35); 14 (2.46±0.09)	7/20
12	8-29-84	20 (2.58±0.19)	11/20
13	9-5-84	4 (<2.35); 16 (2.53±0.12)	11/20
14	9-12-84	19 (2.64±0.21)	16/20
15	9-19-84	1 (<2.35); 19 (2.46±0.10)	9/20
16	9-26-84	19 (2.67±0.24)	6/20
17	10-3-84	19 (2.67±0.21)	2/20
18	10-29-84	19 (2.85±0.24)	6/19
19	11-14-84	2 (2.82±0.28)	8/20
20	12-5-84	2 (<2.35); 18 (2.55±0.14)	8/20
21	12-19-84	2 (<2.35); 17 (2.57±0.12)	12/20
22	1-3-85	3 (<2.35); 16 (2.43±0.06)	10/20
23	1-16-85	4 (<2.35); 15 (2.39±0.04)	11/20

TABLE 16. Serum antibodies against reovirus in day-old progeny from breeders vaccinated with Intervet inactivated reovirus vaccine.

No.	Date of test	ELISA - VA	AGP
1	6-13-84	1 (<2.35); 21 (2.66±0.28)	3/22
2	6-20-84	4 (<2.35); 16 (2.84±0.32)	5/20
3	6-26-84	20 (2.77±0.26)	2/20
5	7-11-84	20 (2.87±0.32)	8/20
6	7-18-84	1 (<2.35); 19 (3.00±0.28)	8/20
7	7-25-84	1 (<2.35); 19 (2.88±0.25)	2/19
8	8-1-84	18 (2.78±0.30)	9/18
9	8-8-84	20 (2.83±0.27)	4/20
10	8-16-84	1 (<2.35); 19 (2.70±0.24)	7/20
11	8-22-84	1 (<2.35); 19 (2.56±0.20)	7/20
12	8-29-84	1 (<2.35); 19 (2.61±0.20)	8/20
13	9-5-84	20 (2.79±0.27)	7/20
14	9-12-84	19 (2.86±0.29)	13/20
15	9-19-84	2 (<2.35); 18 (2.64±0.70)	13/20
16	9-26-84	19 (2.96±0.31)	11/20
17	10-3-84	3 (>3.37); 16 (3.17±0.09)	14/20
18	10-29-84	19 (3.20±0.20)	13/19
19	11-14-84	20 (3.02±0.21)	12/20
20	12-5-84	2 (<2.35); 18 (2.66±0.18)	8/20
21	12-19-84	19 (2.77±0.21)	13/20
22	1-3-85	19 (2.66±0.17)	14/20
23	1-16-85	7 (<2.35); 12 (2.47±0.13)	10/20

TABLE 17. Serum antibodies against reovirus in day-old progeny from non-vaccinated breeders.

No.	Date of test	ELISA - VA	AGP
1	6-13-84	20 (<2.35); 4 (2.37±0.02)	0/24
2	6-20-84	13 (<2.35); 6 (2.36±0.01)	0/20
3	6-26-84	6 (<2.35); 16 (2.39±0.04)	0/20
5	7-11-84	6 (<2.35); 14 (2.41±0.06)	0/20
6	7-18-84	8 (<2.35); 12 (2.40±0.03)	0/20
7	7-25-84	14 (<2.35); 6 (2.26±0.02)	0/19
8	8-1-84	10 (<2.35); 8 (2.39±0.05)	0/18
9	8-8-84	13 (<2.35); 7 (2.35±0.005)	0/20
10	8-16-84	14 (<2.35); 6 (2.36±0.01)	0/20
11	8-22-84	4 (<2.35); 16 (2.38±0.03)	0/20
12	8-29-84	1 (<2.35); 19 (2.44±0.06)	0/20
13	9-5-84	8 (<2.35); 12 (2.39±0.05)	0/20
14	9-12-84	7 (<2.35); 11 (2.38±0.04)	0/20
15	9-19-84	18 (<2.35); 2 (2.36±0.02)	0/20
16	9-26-84	1 (>3.37); 18 (2.54±0.18)	0/18
17	10-3-84	19 (2.53±0.08)	0/20
18	10-29-84	19 (2.46±0.07)	0/19
19	11-14-84	2 (<2.35); 18 (2.45±0.06)	0/20
20	12-5-84	5 (<2.35); 15 (2.39±0.04)	0/20
21	12-19-84	5 (<2.35); 14 (2.41±0.05)	0/20
22	1-3-85	16 (<2.35); 3 (2.35)	0/20
23	1-16-85	17 (<2.35); 2 (2.35)	0/19

TABLE 18. Serum antibodies against reovirus in chicks from 3rd hatch of progeny from broiler breeders vaccinated with inactivated reovirus vaccine and non-vaccinated controls.

Treatment	ELISA	AGP	Age (days)
MBL vaccine	2 (<2.35); 18 (2.54 ± 0.10)	6/20	0
	15 (2.55 ± 0.18)	2/15	14
	12 (<2.35); 3 (2.36 ± 0.02)	0/15	21
	8 (<2.35); 2 (2.38 ± 0.03)	0/15	28
Intervet vaccine	20 (2.77 ± 0.26)	2/20	0
	15 (2.74 ± 0.26)	0/15	14
	7 (<2.35); 8 (2.38 ± 0.02)	0/15	21
	8 (<2.35); 5 (2.46 ± 0.09)	1/14	28
Non-vaccinated:	4 (<2.35); 16 (2.39 ± 0.01)	0/20	0
	3 (<2.35); 10 (2.54 ± 0.23)	0/13	14
	12 (<2.35); 3 (2.36 ± 0.01)	0/15	21
	7 (<2.35); 8 (2.42 ± 0.05)	0/15	28

TABLE 19. Serum antibodies against reovirus in chicks from 5th hatch of progeny from broiler breeders vaccinated with inactivated reovirus vaccines and non-vaccinated controls.

Treatment	ELISA	AGP	Age (days)
MBL vaccine	5 (<2.35); 15 (2.43±0.09)	5/20	0
	11 (2.57±0.11)	8/11	7
	1 (<2.35); 11 (2.39±0.05)	1/12	14
	12 (<2.35); 3 (2.36±0.02)	0/15	21
	8 (<2.35); 7 (2.38±0.03)	0/15	28

Intervet vaccine	20 (2.87±0.32)	8/20	0
	1 (<2.35); 11 (2.73±0.29)	0/12	7
	2 (<2.35); 10 (2.43±0.08)	0/12	14
	10 (<2.35); 5 (2.39±0.02)	0/15	21
	10 (<2.35); 6 (2.38±0.02)	0/16	28

Non-vaccinated	6 (<2.35); 14 (2.41±0.06)	0/20	0
	8 (<2.35); 2 (2.38±0.01)	0/10	7
	12 (<2.35)	0/12	14
	12 (<2.35)	0/12	21
	9 (<2.35); 6 (2.38±0.05)	0/13	28

TABLE 20 Serological response of breeder chickens after vaccination with live and inactivated reovirus vaccines*

Date of bleeding	Intervet, inactivated vaccine		MBL, inactivated vaccine		Unvaccinated control flock	
	ACP	ELISA VA titer	ACP	ELISA VA titer	ACP	ELISA VA titer
3-31-85	28/37	37 (3.02±0.37)	26/36	33 (3.05±0.48); 2 (>4.15)	23/36	35 (3.18±0.33); 1 (>4.15)
5-5-85	33/34	21 (3.26±0.45); 13 (>4.15)	31/33	32 (3.21±0.53); 1 (>4.15)	10/33	12 (<2.25); 21 (2.57±0.030)
5-20-85	33/33	27 (3.31±0.51); 6 (>4.15)	32/32	32 (2.98±0.53)	14/33	10 (<2.25); 23 (2.55±0.33)
6-9-85	32/32	32 (3.25±0.38)	28/32	32 (3.16±0.31)	2/31	2 (<2.25); 29 (2.76±0.32)
7-8-85	22/32	28 (3.51±0.46); 4 (>4.20)	25/31	30 (3.11±0.42); 1 (>4.20)	1/31	2 (<2.25); 29 (2.61±0.28)
8-13-85	25/31	30 (3.47±0.44); 1 (>4.20)	26/30	29 (3.09 0.46); 1 (>4.20)	5/30	2 (<2.25); 28 (2.65±0.37)

*Vaccination with live reovirus vaccine (Intervet, Inc) at 8 and 17-18 weeks of age, followed by intramuscular vaccination with inactivated reovirus vaccine at 20 weeks of age.

TABLE 21 Serology. experiments of progeny from reovirus vaccinated breeder chickens using two strains of reovirus

Date	Age (days)	Progeny of Flock 1		Progeny of Flock 2		Progeny of Flock 3	
		ACP	ELISA VA	ACP	ELISA VA	ACP	ELISA VA
1st Hatch:							
6-2-85	1	10/20	3 (<2.25); 11 (2.65±0.30)	2/20	1 (<2.25); 13 (2.73±0.25)	1/20	12 (<2.25); 3 (2.25)
6-10-85	8	1/15	7 (<2.25); 8 (2.32±0.08)	3/15	2 (<2.25); 8 (2.45±0.09)	0/15	15 (<2.25)
6-16-85	14	0/15	15 (<2.25)	0/15	15 (<2.25)	0/15	15 (<2.25)
6-24-85	22	0/15	15 (<2.25)	0/15	11 (<2.25); 4 (2.31±0.05)	0/15	15 (<2.25)
2nd Hatch:							
6-17-85	1	2/17	4 (<2.25); 11 (2.65±0.36)	10/17	1 (<2.25); 14 (2.42±0.13)	0/17	11 (<2.25); 4 (2.37±0.03)
6-24-85	7	1/16	7 (<2.25); 8 (2.39±0.09)	6/15	6 (<2.25); 9 (2.37±0.10)	0/15	15 (<0.25)
7-1-85	14	0/15	15 (<2.25)	0/15	15 (<2.25)	0/15	15 (<2.25)
7-7-85	21	0/15	15 (<2.25)	0/15	15 (<2.25)	0/15	15 (<2.25)
3rd Hatch:							
6-30-85	1	9/15	4 (<2.25); 12 (2.66±0.36)	11/16	3 (<2.25); 12 (2.36±0.10)	0/16	6 (<2.25); 9 (2.34±0.07)
7-7-85	7	1/15	7 (<2.25); 8 (2.32±0.06)	5/15	7 (<2.25); 8 (2.34±0.06)	0/15	15 (<2.25)
7-15-85	15	0/15	15 (<2.25)	0/15	15 (<2.25)	0/15	15 (<2.25)
7-21-85	21	0/15	15 (<2.25)	0/15	15 (<2.25)	0/15	8 (<2.25); 6 (2.30±0.05)
4th Hatch:							
7-14-85	1	4/17	16 (2.68±0.29)	11/17	2 (<2.25); 14 (2.40±0.11)	1/17	12 (<2.25); 4 (2.33±0.02)
7-21-85	7	1/15	7 (<2.25); 8 (2.33±0.08)	0/15	12 (<2.25); 3 (2.25±0.01)	0/15	15 (<2.25)
7-28-85	14	0/15	15 (<2.25)	0/15	14 (<2.25)	0/15	14 (<2.25)
8-4-85	21	0/15	15 (<2.25)	0/15	11 (<2.25)	0/15	12 (<2.25)
5th Hatch:							
7-28-85	1	2/17	12 (2.67±0.26)	3/17	2 (<2.25); 16 (2.38±0.14)	1/17	7 (<2.25); 10 (2.37±0.07)
8-4-85	7	3/15	5 (<2.25); 10 (2.38±0.09)	6/15	8 (<2.25); 7 (2.33±0.04)	1/15	12 (<2.25); 3 (2.44±0.31)
8-11-85	14	1/15	6 (<2.25); 9 (2.46±0.16)	0/15	10 (<2.25); 5 (2.29±0.03)	0/15	11 (<2.25); 3 (2.34±0.05)
8-19-85	22	0/14	14 (<2.25)	0/15	15 (<2.25)	0/14	14 (<2.25)
6th Hatch:							
8-11-85	1	5/17	7 (<2.25); 9 (2.39±0.10)	12/17	7 (<2.25); 9 (2.56±0.26)	0/17	2 (<2.25); 14 (2.34±0.01)
8-18-85	8	1/15	1 (<2.25); 14 (2.40±0.09)	4/15	4 (<2.25); 11 (2.36±0.09)	0/14	12 (<2.25); 2 (2.28±0.02)
8-25-85	14	0/15	14 (<2.25); 1 (2.30)	0/15	15 (<2.25)	0/15	7 (<2.25)
9-2-85	22	0/15	14 (<2.25)	0/15	14 (<2.25)	0/15	9 (<2.25)